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LINKAGE IN MAIZE: ALEURONE AND CHLOROPHYLL FACTORS[1]

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GENETIC linkages or correlations are beginning to confirm the modern chromosome conception of heredity. As the Mendelian analysis of a species reaches a point where the known genetic factors exceed the number of chromosome pairs, certain group relations between the factors should become evident. Comparatively few genetic linkages have been observed in plants, however, probably because the number of Mendelian factors that have been determined is relatively small compared with the number of chromosomes in most species.

Physiological or morphological correlations, on the other hand, are far more common. But in the present state of knowledge they are not classified as genetic and consequently can not be used as material for determining the relationship between any series of heritable factors.

An intensive, Mendelian study of maize is gradually revealing genetic correlations. Although more than thirty definite Mendelian factors have now been determined in this species, linkages are limited in number because of the relatively large number of chromosomes (at least nine pairs).

As early as 1906, Webber noted a general correlation in maize between color in the aleurone layer and in the stamens, glumes and silks. At that time, the genetic constitution of color in the kernels and other parts of the plant was unknown. Consequently the correlation was not analyzed on a factorial basis.

In 1911, Emerson described an apparent linkage between color of cob, pericarp, husks, silks, and anthers in maize. Further evidence (unpublished), however, has led Professor Emerson to prefer a simpler explanation for this phenomenon than linkage.

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[1] Given before the Botanical Society of America at the annual meeting held in New York City, December 28, 1916. Paper No. 58. Department of Plant Breeding, Cornell University, Ithaca, N. Y.

Collins and Kempton (1911) and Collins (1912) have reported a genetic correlation between aleurone color and endosperm texture. In this case one of the pairs of aleurone factors is apparently linked with one pair of endosperm factors (horny and waxy). The data indicate that there is a little less than twenty-five per cent. crossing over.

In a later paper, Collins (1916) describes five character pairs in maize that show apparent genetic correlation. But since these characters exhibit "blending" inheritance and have not been analyzed from any factorial standpoint, it becomes impossible to use these correlations in determining the group relations between the genetic factors concerned. Collins also notes a large number of physiological correlations in this article.

LINKAGE BETWEEN ALEURONE AND CHLOROPHYLL FACTORS

A definite linkage has been found in maize between one of the five pairs of aleurone factors (*Aa, Cc, Rr, Pp, Ii*)[2] and one pair of chlorophyll factors of which there are at least seven, as the writer has determined. Manifestly this is not a physiological correlation, for it is difficult to conceive of an anthocyanic pigment, limited to a single layer of cells in the grain, being caused by the same physiological factor that produces a plastid color, like chlorophyll, so widely distributed in the plant. Breeding evidence demonstrates that the correlation is genetic, as will be seen later.

The aleurone factor concerned is the *R* factor, which together with *C* and *A* is needed to produce any color in the aleurone cells of the corn grain (Emerson, 1917). To determine linkages with any of the five aleurone factors, it is obviously desirable to use material that is homozygous for as many of the non-linked factors as possible. Fortunately, the plants used in this experiment were homozygous for *A, P* and *i,* as will be shown later. This left only *C* and *R,* giving either 3:1 or 9:7 ratios of purple to colorless grains. The aleurone segregation on the ear proved to be exceptionally distinct because the factors *A* and *P* were homozygous.

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[2] Reported in a paper given before the Botanical Society of America at the annual meeting in New York City, December 28, 1916, by Professor R. A. Emerson. The paper is now in manscript form under the title, "A Fifth Pair of Factors for Aleurone Color in Maize."

The other factor involved in the linkage is concerned with chlorophyll development in the mature plant. It is one of at least seven factors necessary for the production of full, normal green color in maize, and it has been termed the *G* factor. Its allelomorph *g* produces a distinct yellow or golden color in the leaves and stalk of the mature corn plant. This color is comparable with that of the familiar golden-leaved shrubs. It has been described and its inheritance discussed by Emerson (1912) and Miles (1915). Suffice it to say that it is a simple recessive to normal green.

During the summer of 1914, a green plant heterozygous for *R* and *G* was pollinated by a golden plant that lacked aleurone color. The cross can best be described by the following factors:

$$\frac{GgRrCCAAPPii}{3472\ (11)} \times \frac{ggrrCcAAPPii}{3468\ (10)}$$

Proof for the correctness of these formulæ will be given later.

A selfed ear of the female parent bore a 3:1 ratio (271:88) of purple to colorless grains, showing that only one aleurone factor was heterozygous. The male parent was likewise selfed and showed no aleurone color. On the ear of this cross there were 67 purple and 55 colorless grains, approximating a 1:1 ratio.

The purple seeds on this ear were planted separately from the colorless seeds. From the field counts of this planting, which are given in Table I, it is seen that the 1:1:1:1 ratio of independent inheritance is noticeably modified.

The observed numbers in the four classes give a gametic ratio of 3.9 : 1, or 20.4 per cent. of crossovers. It is evident that the actual results agree very closely with the theoretical expectancy on a 4 : 1 basis of linkage. Indeed, the goodness of fit (Elderton, 1901 and Harris, 1912) is so perfect that $x^2 = .317$, giving a very high value for *P*.

TABLE I

SHOWING THE F_1 DISTRIBUTION FROM THE CROSS

$$\frac{GgRrCC}{3472\ (11)} \times \frac{ggrrCc}{3468\ (10)}.$$

	From Purple Seeds		From Colorless Seeds	
	Green (*RG*)	Golden (*Rg*)	Green (*rG*)	Golden (*rg*)
Field counts..............................	30	9	6	28
Observed, corrected for aleurone ratio[3]	28.1	8.4	6.5	30 0
Theoretical, on a 4 : 1 gametic ratio...	29.2	7.3	7.3	29.2

From these results it may be said that, at gametogenesis, the factors of the female parent are so linked that the gametes *RG* and *rg* are produced about four times as often as the crossover gametes *Rg* and *rG*. Thus far, no evidence is available for demonstrating the linkage of *Rg* and *rG* ("repulsion" between *R* and *G*).

Additional evidence on the linkage was afforded when the female parent of the cross and four other plants, in which the *R* and *G* factors were heterozygous, were selfed. Each of these plants bore ears with 3 : 1 ratios in aleurone color (total, 1052 purple : 339 colorless). The results appear in Table II.

Obviously this F_2 distribution does not resemble a 9 : 3 : 3 : 1 ratio of independent inheritance. The agreement between the actual results and the theoretical on a 4 : 1 gametic ratio, however, is close, *P* being .6733, which is considered a good fit. It may be concluded, therefore, that the factors in these five F_1 plants were so linked that *R* and *G* occurred together in one chromosome, while *r* and *g* were located in the homologous chromosome and that crossing over occurred about twenty per cent. of the time.

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[3] This correction is applied to equalize the proportion of the plants from the purple and the colorless seeds. The proportion should be 1 : 1 in this case, but variation in the percentage of germination or in the number of purple and colorless seeds actually planted, often disturbs it. Such a correction is of course legitimate.

TABLE II

SHOWING THE F_2 DISTRIBUTION WHEN F_1 PLANTS HETEROZYGOUS FOR *R* AND *G* WERE SELFED

Selfed Plants	From Purple Seeds		From Colorless Seeds	
	RG	*Rg*	*rG*	*rg*
3472 (11)	48	4	12	24
3347 (2) parent of above	35	3	8	6
3954 (1)	5	0	2	2
3954 (3)	10	0	2	3
3954 (11)	8	3	2	6
Total	106	10	26	41
Correction for aleurone ratio[4]	125	12	18	28
Theoretical, 4 : 1 basis	120.8	16.5	16.5	29.2

When plants that were heterozygous for *G* and for both *R* and *C* were selfed, the ratio between the four classes in the next generation appeared rather unusual. This was found to be due to the influence of the 9:7 ratio in aleurone color of each of the four plants that were selfed (total, 477 purple:387 colorless). The data from such plants are arranged in Table III, which follows:

TABLE III

SHOWING F_2 DISTRIBUTION WHEN PLANTS HETEROZYGOUS FOR *G, R* AND *C* WERE SELFED

Plants Selfed	From Purple Seeds		From Colorless Seeds	
	Green	Golden	Green	Golden
3347 (1)	32	3	11	4
3347 (3)	79	6	51	31
3347 (4)	96	6	47	40
3477 (2)	38	3	19	11
Total	245	18	128	86
Theoretical 4 : 1 basis	236	32	122	87

There is considerable deviation from the theoretical expectancy in this case and the value for *P* is small ($P = .0808$). Nevertheless, these results, taken in conjunction with those in Tables I and II, accord with the idea of linkage between R and G on a 4:1 basis, especially since they deviate so widely from the 27:9:21:7 ratio of independent inheritance.

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[4] The correction is applied because at planting time no attempt was made to plant three purple grains to one colorless grain.

Another source of evidence on the genetic interrelations of the R and G factors was noted in a back cross in which the I factor for aleurone color was involved. No aleurone tests have been made, but evidence from related plants in pedigree cultures makes it seem reasonable that the following factors are concerned:

$$\frac{GgRrccAAPPIi}{3021\ (1)} \times \frac{ggrrCcAAPPii}{3018\ (10)}.$$

In this case, the ears of both parents showed no aleurone color. The ear from the cross gave a distinct segregation of purple and of colorless grains (36 purple:195 colorless), approximating the theoretical 1:7 ratio. Assuming the factors as given above, this aleurone ratio is reasonably close to the expected proportion, the numbers being relatively small. As a check upon this aleurone ratio, it might be mentioned that six of the F_1 plans from purple seed were selfed. Each one showed a 9:7 ratio of purple to colorless grains on the ear (total, 1441 purple:1088 colorless). Also three F_1 plants from colorless seed were selfed and the ears showed no aleurone color.

The field counts of the plants from purple and from colorless F_1 grains are classified in the following table:

TABLE IV

Showing the F_1 Distribution from the Cross

$$\frac{GgRrccAAPPIi}{3021\ (1)} \times \frac{ggrrCcAAPPii}{3018\ (10)}.$$

	From Purple Seeds		From Colorless Seeds	
	Green	Golden	Green	Golden
Observed	12.0	3.0	8.0	22.0
Correction for aleurone ratio	4.5	1.1	10.4	29.0
Theoretical, 4:1 basis	4.5	1.1	18.0	21.4

Here again, while the value for P is relatively small ($P = .1171$), it is noted that in general the observed results correspond with the theoretical when a linkage on a 4:1 basis is assumed. Considering only the classes from purple seeds (first two columns in Table IV), the agreement is perfect. These classes are not disturbed by the aleurone situation and consequently ought to show the gametic ratio directly.

Determination of the Aleurone Factor concerned in the Linkage

It has been shown from four independent sources that the *G* factor is linked with one of the five aleurone factors, which has been termed the *R* factor. It now becomes necessary to prove that it really is the *R* factor that is involved. From the aleurone ratios observed in this experiment, as well as from evidence from related plants, it is certain that the inhibiting factor *I* is lacking, with the exception of the last cross described (Table IV). It is also obvious that the *P* factor, which produces purple color when *C, R* and *A* are present, must be homozygous (*PP*) in both parents of the first cross since the color in the colored grains is a deep purple with no trace of red. This leaves only *C, R* and *A* to be considered.

In order to facilitate the presentation of the proof that the *R* factor is the one concerned, Table V has been prepared. In this table, the zygotic formulæ of the F_1 plants of the first back cross (see Table I) are listed and the plants that were tested are noted in the last column. The factors are merely assumed as here given.

TABLE V

Showing the Zygotic Formulæ of the F_1 Plants of the Cross 3472 (11) × 3468 (10)

Parental Formulæ: GgRrCCAA × ggrrCcAA

F_1 Zygotes	F_1 Aleurone Color	F_1 Plant Type	F_1 Plants Selfed (Pedigree No. 3954) Individual Plant Nos.
GgRrCCAA	Purple	Green	(1) (3) (11) (31) (33)
GgRrCcAA	"	"	(34)
ggRrCCAA	"	Golden	
ggRrCcAA	"	"	(32)
GgrrCCAA	Colorless	Green	(44)
GgrrCcAA	"	"	
ggrrCCAA	"	Golden	(21) (22) (29) (43) (45)
ggrrCcAA	"	"	

The fourth column shows the plant numbers of the F_1 plants, which were self-fertilized. In addition, plants (31), (32) and (34) were crossed with certain aleurone testers of known constitution provided by Professor R. A. Emerson.

All the grains on the ears of plants (21), (22), (29), (43), (45) and (44) were colorless. The aleurone counts on the ears from purple seed are arranegd in Table VI.

TABLE VI

SHOWING THE ALEURONE COUNTS ON SELFED EARS OF F_1 PLANTS FROM THE CROSS 3472 (11) × 3468 (10)

Ped. 3954 Plant No.	3 : 1 Ears		9 : 7 Ears		
	Purple	Colorless	Ped. 3954 Plant No.	Purple	Colorless
(1)	230	83	(32)	66	65
(3)	303	88	(34)	172	163
(11)	248	80			
(31)	180	45			
(33)	224	79			
Total	1,185	375	Total	238	228
Theoretical	1,170	390	Theoretical	262	204

These ratios indicate that plants (1), (3), (11), (31) and (33) are heterozygous for one aleurone factor only. Plants (32) and (34) also contain that factor but apparently they have in addition another aleurone factor, which likewise is heterozygous because the aleurone ratios, although they deviate somewhat from the 9:7 proportion, can not be classified as 3:1 or 27:37 ratios.

Only tests with plants of known aleurone formulæ will determine whether it is the *C*, *R*, or *A* factor that is linked with *G*. Such tests have been made by using the aleurone testers described by Emerson (1917). These aleurone testers possess colorless grains in which all the aleurone factors except *P* are homozygous. For example, the *R* tester has the formula *rrCCAAii*, the *C* tester the formula *RRccAAii*, and the *A* tester the formula *RRCCaaii*. In Table VII are presented the data involving the various tests for the aleurone factors. Plants (31), (32) and (34) possess all the three aleurone factors in question

(*R, C,* and *A*), as can be seen from their records in Table VI. Plant (41) is a golden type from colorless seed.

TABLE VII

SHOWING THE TESTS FOR THE PRESENCE OF ALEURONE FACTORS

Crosses with Aleurone Testers	Aleurone Color of F_1 Grains
1. *A* tester (*RRCCaa*) × 3954 (32)	450 purple
2. *C* tester (*RRccAA*) × 3954 (32)	72 " : 69 colorless
3. *R* tester (*rrCCAA*) × 3954 (32)	7 " : 11 "
4. 3954 (34) × *C* tester (*RRccAA*)	205 " : 217 "
5. 3954 (31) × *R* tester (*rrCCAA*)	212 " : 249 "
6. 3954 (41) (*rrCcAA*) × 3954 (31)	92 " : 101 "

The data in Table VII prove several things, namely:

1. That the factors *A, C* and *R* must be present in all the plants tested, because color was produced in some of the F_1 grains of each cross.

2. That it is the *R* factor that is linked with *G*. This is demonstrated in the fifth cross. Plant (31) was a green plant from purple seed bearing an ear with a 3:1 aleurone ratio, showing that it was heterozygous for the one aleurone factor that is involved in the linkage. The test clearly proves it to be the *R* factor, the aleurone ratio from the fifth cross approximating the theoretical 1:1 proportion.

3. That the assumptions made in Table V as to the genotypic formulæ of the F_1 plants are correct. This statement follows from a series of deductions. First, one aleurone factor of the three concerned must be homozygous (dominant) in all the plants, because the aleurone ratios observed permit only two heterozygous factors at the most (9:7 ratios). Since the *C* factor is heterozygous in plants (32) and (34), and the *R* factor heterozygous in plant (31), while *A* is homozygous in plant (32), it is obvious that only the *A* factor could be homozygous (dominant) in all the F_1 plants. Second, one of the remaining factors (*C* or *R*) must be homozygous (dominant) in some plants and heterozygous in others to account for the 3:1 and the 9:7 ratios, respectively. The second and fourth crosses in Table VII indicate that *C* is heterozygous in plants (32) and (34), whereas the fifth

cross, together with the 3:1 ratio of the selfed ear, shows that *C* is homozygous in plant (31). Third, the remaining factor, *R*, should occur only in a heterozygous or in a homozygous recessive condition to account for the 1:1 aleurone ratio on the F_1 ear of the original back cross. The fifth cross in Table VII proves that *R* is heterozygous in plant (31), and the sixth cross shows that in plant (41) this same factor is homozygous recessive.

From this series of interrelations it is seen that the hypothesis is verified in all cases and that it is the *R* factor for aleurone that is linked with *G*.

ON THE QUESTION OF CROSSING OVER IN THE MALE AND FEMALE

An interesting observation regarding the question of crossing over in plants can be derived from some of the data presented. It has been shown that crossing over occurs in gametogenesis of the female (Table I). Does it take place in the formation of the male gametes as well? In certain animals, crossing over seems to be limited to one sex. It occurs only in the female of *Drosophila* (Morgan, 1915) and only in the male of the silkworm (Tanaka, 1914). Castle (1916) has noted that the phenomenon occurs in both sexes of the rat. Among plants, the studies with sweet peas and Primula indicate that crossing over is not restricted to one sex. Perhaps it is to be expected that, in the case of most plants, where the pistillate and staminate parts are borne on the same individual, there should be no difference in the genetic behavior of the two reproductive systems in this respect. Nevertheless, it is interesting to note the condition in the monecious corn plant.

From the data in Table II, it is possible to demonstrate that crossing over is found in both sexes of maize. In order to do this, the observed frequencies can be compared with the theoretical expectation when crossing over occurs in both sexes and when it takes place only in the female. Such a comparison is arranged in Table VIII, which follows:

TABLE VIII

A COMPARISON OF THE RESULTS FROM TABLE II WITH THE THEORETICAL EXPECTATION WHEN CROSSING OVER OCCURS IN BOTH SEXES AND WHEN IT OCCURS ONLY IN THE FEMALE

	RG	*Rg*	*rG*	*rg*	*P* Goodness of Fit as Compared with Observed
Observed (from Table II)	125	12	18	28	
Theoretical, when crossing over occurs in both sexes	120.8	16.5	16.5	29.2	.6733
Theoretical, when crossing over occurs only in the female	128.0	9.0	9.0	37.0	.0067

Clearly, the first theoretical expectation fits the case adequately, for with such a high value for *P* it is almost certain that the deviations of the observed results from the theoretical are due to errors of random sampling only. Consequently one is justified in saying that crossing over occurs in both male and female. This is especially true when the fit in the second case is so poor. In fact, the great difference between the two values for *P* makes it seem reasonable that the intensity of the linkage is equal in both male and female, although the high value for *P* in the first case suggests that directly.

ADDITIONAL LINKAGES BETWEEN CHLOROPHYLL FACTORS AND ALEURONE

Preliminary tests indicate that the same chlorophyll factor, *G,* that is linked with *R,* is also concerned in a linkage with one of the other chlorophyll factors termed *L.* The latter has been found to be one of three factors, two of which have already been described by Miles (1915), involved in the production of chlorophyll in the seedling stage of maize.

Factors *G* and *L* seem to be linked although the data from three back crosses, in which the numbers are small, exhibit some variation in the percentage of crossing over. Details of this linkage will appear in a later paper, dealing with the inheritance of the three seedling chlorophyll factors.

Apparently, then, the factor pairs *Rr, Gg,* and *Ll* constitute one factorial group in maize. It is to be expected that *Rr* and *Ll* should bear a definite relationship to one another. This has not yet been fully determined, although there are some indications of such a linkage, for aleurone color and chlorophyll development appear to be genetically related in different manner from that noted previously.

When purple seed of certain ears are planted separately from the colorless ones, the former give a distinct segregation of green and white seedlings, while the latter give rise not only to the green and white, but also to a constant proportion of yellow seedlings. The writer has determined that these yellow seedlings depend upon a definite genetic factor. Over two thousand seedlings have now been grown and not one yellow seedling has resulted from the purple grains. Discussion of this linkage will also be reserved for the later paper, or until the aleurone factor concerned has been identified. This may be the *R* factor showing its theoretical relationship to the *L* factor.

SUMMARY

1. Linkage between the *R* aleurone factor and the *G* factor for chlorophyll development shows approximately 20 per cent. crossovers.

2. Crossing over takes place in both male and female gametogenesis of the monecious maize plant.

3. Preliminary tests indicate that *G* is also linked with *L,* a seedling, chlorophyll factor. Consequently the factor pairs *Rr, Gg* and *Ll* constitute one factorial group in maize.

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